

Preliminary Amendment
Takeshi OKUDA et al.

U.S. Patent Application S.N. 09/901,572
Attorney Docket No. 010898

Please replace the paragraph beginning on page 14, line 32, with the following rewritten paragraph:

As the gene region nonessential for the growth of poxvirus, there can be used the TK gene region of fowlpox virus, pigeon poxvirus, quail pox (?), turkey poxvirus etc., the region in between the open reading frames (ORF) as described in U.S. Pat. No. 5,180,675 and Japanese Patent Publication No. 2766984, and the region described in U.S. Pat. No. 5,387,519. More specifically, an EcoRI fragment (7.3 kbp), an EcoRI-HindIII fragment (about 5.0 kbp), a BamHI fragment (about 4.0 kbp), and a HindIII fragment (about 5.2 kbp) derived from pigeon pox described in U.S. Pat. No. 5,387,519, and SpeI-HpaI (3026 bp) (FPV-29 insertion site) in an EcoRI fragment (7.3 kbp).

Please replace the two paragraphs beginning on page 19, line 20, with the following rewritten paragraphs:

The DNA sequences corresponding to two N-glycosylation sites present in a 186 bp (62 amino acids) signal sequence of Marek's disease virus gB protein (MDV gB signal) were modified based on the above principle of alteration A, and DNA encoding the amino acid sequence (modified MDV gB signal) was obtained. The DNA was further modified based on the above principle of alteration B and C to obtain the MDVgB signal DNA (modified MDV gB-signal) in which the 30 nucleotide sequence was modified.

Please replace the three paragraphs beginning on page 19, line 37, with the following rewritten paragraphs:

DNA encoding the rabies virus gG signal (23 amino acids) (Rabies virus glycoprotein) G(gG signal) having no

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N-glycosylation sites was modified based on the above principle of alteration B-D to obtain a plasmid pUC-rgG that has an modified rabies gG signal DNA (Modified rabies glycoprotein G signal).

Specific procedure to obtain this plasmid is as follows:

Thus, after annealing two synthetic DNAs (synthetic DNA-1:1 BN for Rabies gG and synthetic DNA-2: 1BC for Rabies gG), it was inserted into a 2665 bp BamHI-EcoRI-cleaved fragment of pUC18 to construct pUC-rgG.

Please replace the paragraph beginning on page 20, line 14, with the following rewritten paragraph:

Act
There are four N-glycosylation sites in the TTM-1 portion of the amino acid sequence as set forth in SEQ ID NO: 12 3 of a plasmid pNZ40K-S described in International Patent Publication W097/36924 in which a MDVgB signal-encoding DNA has been ligated to the N-terminal of the antigen gene TTM-1 derived from *Mycoplasma gallisepticum*. Since there are no N-glycosylation sites in the region from the EcoRI site, the start of the TTM-1 portion, to the BglII site 83 bp downstream, the portion downstream of BglII was modified based on the above principle of alteration A to obtain a plasmid pGTPs40KS-Ngly having an modified TTM-1 gene in which the sequence downstream to the BglII site has the nucleotide sequence (TTM-1 portion of the modified pNZ40K-S (downstream of BglI)) that corresponds to the amino acid sequence. The specific procedure to obtain this plasmid is as follows:

Please replace the six paragraphs beginning on page 21, line 2, with the following rewritten paragraphs:

Act
First, PCR was performed with the primer 40KG-1 and the primer 40KG-2R to obtain a 136 bp

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fragment.

Similarly, PCR was performed with the primer 40KG-2 and the primer 40KG-3R to obtain a 341 bp fragment.

Similarly, PCR was performed with the primer 40KG-3A and the primer 40KG-4RA to obtain 190 bp fragment.

Similarly, PCR was performed with the primer 40KG-4 and the primer 40KG-5R to obtain a 359 bp fragment.

Similarly, PCR was performed with the primer 40KG-5 and the primer 40KF-6R to obtain a 218 bp fragment.

In the next PCR, using three fragments of a 136 bp RCE product of the primers 40KG-1 and 40KG-2R, a 341 bp PCR product of the primers 40KG-2 and 40KG-3R, and a 190 bp PCR product of the primers 40KG-3A and 40KG-4RA as the templates, PCR was performed in the above-mentioned condition with the primer 40KG-1 and the primer 4KG-4RA to obtain a 595 bp fragment.

Please replace the paragraph beginning on page 21, line 25, with the following rewritten paragraph:

Similarly, using a 359 bp PCR product of the primers 40KG-4 and 40KG-5R and a 218 bp PCR product of the primers 40KG-5 and 40KG-6R as the templates, PCR was performed in the above-mentioned condition with the primer 40KG-4 and the primer 4KG-6R to obtain a 539 bp fragment.

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Please replace the paragraph beginning on page 21, line 31, with the following rewritten paragraph:

Furthermore, using a 595 bp PCR product of the primers 40KG-1 and 40KG-4RA and a 539 bp PCR product of the primers 40KG-4 and 40KG-6R as the templates, PCR was performed in the above-mentioned condition with the primer 40KG-1 and the primer 4KG-6R to obtain a partial fragment (1088 bp) of the modified TTM-1.

Please replace the paragraph beginning on page 22, line 8, with the following rewritten paragraph:

The amino acid sequence as set forth in SEQ ID NO: 4 encoded by the mgc3 gene (GeneBank accession No. AB023292) as set forth in SEQ ID NO: 2 has 16 N-glycosylation sites. Among the 16 sites, since a site at the most 5' upstream side is replaced with the signal sequence at a later treatment, it was excluded from the target of alteration. For the remaining 15 sites, the base sequence was modified based on the principle of alteration A to obtain the plasmid pMllBTR containing the mgc3 gene that has the nucleotide sequence (modified mgc 3 gene (M11-BTR)) corresponding to the amino acid sequence (modified MG3g antigen (M11-BTR)).

Please replace the six paragraphs on page 23, line 3, with the following rewritten paragraphs:

Using pUC-MGC3 as the template, PCR was performed with the primer Mll-B and the primer M11-2R to obtain a 136 bp fragment.

Using pUC-MGC3 as the template, PCR was performed with the primer Mll-2 and the primer Mll-3R

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to obtain a 92 bp fragment.

Using pUC-MGC3 as the template, PCR was performed with the primer M11-3 and the primer M11-4RB to obtain a 271 bp fragment.

Using pUC-MGC3 as the template, PCR was performed with the primer M11-4B and the primer M11-5R to obtain a 116 bp fragment.

Using pUC-MGC3 as the template, PCR was performed with the primer M11-5 and the primer M11-7RA to obtain a 439 bp fragment.

Using pUC-MGC3 as the template, PCR was performed with the primer M11-7 and the primer M11-2KR to obtain a 201 bp fragment.

Please replace the paragraphs beginning on page 23, line 21, through page 26, line 33, as follows:

In the next PCR, using a 136 bp PCR product of the primer M11-B and the primer M11-2R and a 92 bp PCR product of the primer M11-2 and the primer M11-3R as the templates, PCR was performed with the primer M11-B and the primer M11-3R to obtain a 199 bp fragment.

Similarly, using a 271 bp PCR product of the primer M11-3 and the primer M11-4RB and a 116 bp PCR product of the primer M11-4B and the primer M11-5R as the templates, PCR was performed with the primer M11-3 and the primer M11-5R to obtain a 344 bp fragment.

Similarly, using a 439 bp PCR product of the primer M11-5A and the primer M11-7RA and a 201 bp PCR product of the primer M11-7 and the primer M11-KR as the templates, PCR was performed with the primer M11-5A and the primer M11-KR to obtain a 610 bp fragment.

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Using these three PCR products, a PCR product (199 bp) of the primer M11-B and the primer M11-3R, and a PCR product (361 bp) of the primer M11-3 and the primer M11-5R, and a PCR product (610 bp) of the primer M11-5A and M11-KR as the templates, PCR was performed with the primer M11-B and the primer M11-KR to obtain a 1094 bp fragment. A 1070 bp fragment obtained by cleaving this PCR product (1094 bp) 10 of the primers M11-B and M11-KR with EcoRI and KpnI was inserted into a 2678 bp fragment of the plasmid pUC18 cleaved with EcoRI and KpnI to construct pM11BKR.

After analyzing the nucleotide sequence of this pM11BKR, a sequence different from the mgc3 gene (GeneBank accession No. AB023292) registered in GeneBank was found. Furthermore, the sequence of the original plasmid pUC-MGC3 was compared to confirm that it was not an error in PCR. As a result, it was confirmed that G at position 308 of SEQ ID NO: 2, a sequence registered at GeneBank, is T, G at position 311 is C, C at position 561 is G, and G at position 749 is T. It was demonstrated that due to an error in the base at position 561, the amino acid sequence encoded in this region is not N-Asn(N)-Gln(Q)-Thr(T) corresponding to the N-glycosylation site but Gln(Q)-Gln(Q)-Thr(T).

(2) Mutation of the KXR region (construction of pM11KXR)

Using pUC-MGC3 as the template, PCR was performed with the primer M11-K and the primer M11-8R to obtain a 151 bp fragment.

Using pUC-MGC3 as the template, PCR was performed with the primer M11-8 and the primer M11-10R to obtain a 109 bp fragment.

Using pUC-MGC3 as the template, PCR was performed with the primer M11-10 and the primer M11-12RA to obtain a 416 bp fragment.

Using pUC-MGC3 as the template, PCR was performed with the primer M11-12A and the primer

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M11-XR to obtain a 350 bp fragment.

In the next PCR, using a 109 bp PCR product of the primer M11-8 and the primer M11-10R and a 416 bp PCR product of the primer M11-10 and the primer M11-12RA as the templates, PCR was performed with the primer M11-8 and the primer M11-12RA to obtain a 487 bp fragment.

Furthermore, using a 151 bp PCR product of the primer M11-K and the primer M11-8R and a 487 bp PCR product of the primer M11-8 and the primer M11-12RA as the templates, PCR was performed with the primer M11-K and the primer M11-12RA to obtain a 596 bp fragment.

Using the above two PCR products, a 596 bp PCR product of the primer M11-K and the primer M11-12RA and a 350 bp PCR product of the primer M11-12A and the primer M11-XR as the templates, PCR was performed with the primer M11-K and the primer M11-XR to obtain a 908 bp fragment.

A 885 bp fragment obtained by cleaving this PCR product (908 bp) of the primer M11-K and the primer M11-XR with Kpn1 and Xba1 was inserted into the plasmid pUC18 cleaved with Kpn1 and Xba1 to construct pM11KXR.

After analyzing the nucleotide sequence of this pM11KXR, a sequence different from the *mgo3* gene (GeneBank accession No. AB023292) registered in GeneBank was found. Furthermore, the sequence of the original plasmid pUC-MGC3 was compared to confirm that it was not an error in PCR. As a result, it was confirmed that G at position 1279 of SEQ ID NO: 2, a sequence registered at GeneBank, is A, T at position 1729 is G, and C at position 1732 is G.

(3) Mutation of the XGTR region (construction of pM11XGTR)

Using pUC-MGC3 as the template, PCR was performed with the primer M11-XA and the primer M11-13RA to obtain a 238 bp fragment.

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Using pUC-MGC3 as the template, PCR was performed with the primer M11-13A and the primer M11-14RA to obtain a 266 bp fragment.

Using pUC-MGC3 as the template, PCR was performed with the primer M11-14A and the primer M11-15RA to obtain a 168 bp fragment.

Using pUC-MGC3 as the template, PCR was performed with the primer M11-15A and the primer M11-16RA to obtain a 123 bp fragment.

Using pUC-MGC3 as the template, PCR was performed with the primer M11-16A and the primer M11-GTR to obtain a 556 bp fragment.

In the next PCR, using a 238 bp PCR product of the primer M11-XA and the primer M11-13RA and a 266 bp PCR product of the primer M11-13A and the primer M11-14RA as the templates, PCR was performed with the primer M11-XA and the primer M11-14RA to obtain a 463 bp fragment.

Furthermore, using a 168 bp PCR product of the primer M11-14A and the primer M11-15RA and a 123 bp PCR product of the primer M11-15A and the primer M11-16RA as the templates, PCR was performed with the primer M11-14A and the primer M11-16RA to obtain a 253 bp fragment.

Using the above three fragments of a 463 bp PCR product of the primer M11-XA and the primer M11-14RA, a 253 bp PCR product of the primer M11-14A and the primer M11-16RA, and a 556 bp PCR product of the primer M11-16A and the primer M11-GTR as the templates, PCR was performed with the primer M11-XA and the primer M11-GTR to obtain a 1192 bp fragment.

Please replace the paragraphs beginning on page 27, line 22, as follows:

A 2676 bp fragment obtained by cleaving, with HindIII and SalI pUC18XG described in International

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Patent Publication W099/18215 in which the restriction sites of pUC18 were modified and an annealing product of a 5'-end-phosphorylated linker H'-S-H-S-P-S1 and a linker H'-S-H-S-P-S2 were ligated to construct pUCSfi-H-S.

(2) Construction of pGHPs

Three fragments, a 2661 bp fragment obtained by cleaving pUCSfi-H-S with HindIII and EcoRI, an annealing product of a 5'-end-phosphorylated linker S-B-E1 and a linker S-B-E2, and a 137 bp fragment obtained by cleaving, with HindIII and SalI, the plasmid described in International Patent Publication WO97/36924 containing the poxvirus late and early promoter, were ligated to construct a plasmid pGHPs.

Please replace the paragraphs beginning on page 29, line 32, through page 30, line 15, as follows:

Using pNZ29RMG40KM11CS-G constructed in Example 4(2) as the template, PCR was performed with the primer M11-Sfi and the primer M11-5RB in the condition described in Example 1 to obtain a 836 bp fragment.

Using pNZ29RMG40KM11CS-G constructed in Example 4(2) as the template, PCR was performed with the primer M11-5C and the primer M11-KRA in a similar condition to obtain a 618 bp fragment.

Using these two PCR products as the templates, PCR was performed with the primer M11-Sfi and the primer M11-KRA to obtain a 1400 bp fragment. A 1368 bp fragment obtained by cleaving this 1400 bp PCR product with SfiI and KpnI and a 9032 bp fragment obtained by cleaving pNZ29RMG40KM11CS-G constructed in Example 4(2) with SfiI and KpnI were ligated to construct a plasmid pNZ29RMG40KMHCS-G2 having the modified MDVgB signal DNA, the modified TTM-1 gene, and the re-modified mgc3 gene.

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Please replace the paragraphs beginning on page 30, line 34, through page 32, line 1, as follows:

Using pGIPec described in Japanese Unexamined Patent Publication (Kokai) No. 2001-188 as the template, PCR was performed in the condition created in Example 1 with the primer pCMV-1 and the primer pPeclR to obtain a 293 bp fragment.

Using pBK-CMV (Stratagene) as the template, PCR was performed in a similar condition with the primer pCMV-ol and the primer CMV-R1 to obtain a 341 bp fragment.

Using the two PCR products obtained as the templates, PCR was performed with the primer pCMV-1 and the primer pCMV-R1 to obtain a 604 bp fragment.

A 589 bp fragment obtained by cleaving this 604 fragment with PstI and XbaI and a 2765 bp fragment obtained by cleaving pGIPec with PstI and XbaI were ligated to construct pGICMV(-).

(4) Construction of pGHMCSpolyASfi

Using the plasmid pGIMCSpolyASfi described in International Patent Publication W099/18215 having the polyA signal of UL46h, UL47h and UL49h and a multiple cloning site as the template, PCR was performed with the primer pGHP-F and the primer pGHP-R to obtain a 149 bp fragment.

A 138 bp fragment obtained by cleaving this 149 bp fragment with EcoRI and HindIII and a 2635 bp fragment obtained by cleaving pGIMCSpolyASfi with EcoRI and HindIII were ligated to obtain pGHMCSpolyASfi.

(5) Construction of pGHCMV(-)

A 2765 bp fragment obtained by cleaving pGHMCSpolyASfi obtained in the above (4) with PstI and XbaI and a 589 bp fragment obtained by cleaving pGICMV(-) obtained in the above (3) with PstI and XbaI were ligated to construct pGHCMV(-).

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(6) Construction of pHCMV(-)

A 3294 bp fragment obtained by cleaving pGHCMV(-) obtained in the above (5) with EcoRI and Sait and an annealing product of 5'-end-phosphorylated Linker 1 and a Linker 2 were ligated to construct a plasmid pHCMV(-) in which the polyA signal of UL46h, UL47h, and UL49h of MDV-1 was deleted in pGHCMV(-).

Please replace the paragraphs beginning on page 32, line 24, through page 33, line 11, as follows:

Using pBK-CMV (Stratagene) as the template, PCR was performed with the primer PolyA-SfiF2 and the primer PolyA-SalKpn to obtain a 313 bp fragment having the PolyA signal of SV40. A 297 bp fragment obtained by cleaving this 313 bp fragment with SfiI and KpnI and a 4222 bp fragment obtained by cleaving pBAC(dHS) obtained in the above (9) with SfiI and KpnI were ligated to construct pGIBacpA2nd.

(11) Construction of pGIBac40KS2nd

Using pGTPs40K-S described in International Patent Publication W097/36924 as the template, PCR was performed with the primer 40KS-B and 40KG-6R to obtain a 1359 bp fragment. A 1345 bp fragment obtained by cleaving this 1359 bp fragment with BamHI and SalI and a 4222 bp fragment obtained by cleaving pGIBacpA2nd obtained in the above (10) with BamHI and SalI were ligated to construct a plasmid pGIBac40KS2nd.

(12) Construction of p45/46Bac40KS+2nd

A 3169 bp fragment obtained by cleaving pGIBac40KS2nd obtained in the above (11) with BgII was inserted into the SfiI site of pNZ45/46Sfi having the UL44 to UL46 region (HVT-UL44-46 insertion site) of HVT described in W097/36924 to construct a plasmid p45/46Bac40KS+2nd having the TTM-1 gene.

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Please replace the paragraph beginning on page 33, line 33, with the following rewritten paragraph:

Using pUTTM-1 described in U.S. Pat. No. 5,489,430 as the template, PCR was performed with primer pMG40K-1 and the primer 40KG-6R to obtain a 1259 fragment. A 1237 bp fragment obtained by cleaving this 1259 bp fragment with BamHI and Sall and a 7317 bp fragment obtained by cleaving pNZ45/46BacpA2nd obtained in the above (15) with BamHI and Sall were ligated to construct a plasmid pNZ45/46Bac40KpA+2nd.

Please amend pages 55, line 1 through page 59, line 4, as follows:

Sequence listing Free text

Sequence No. 1 TTM-1 gene (behind EcoRI)

Sequence No. 2 mgc3 gene

Sequence No. 3 TTM-1 portion of pNZ40K-S (amino acid)

Sequence No. 4 MGC3 protein encoded by the mgc3 gene (amino acid)
